Lysis of Yeast Cell Walls Induced by 2-Deoxyglucose at Their Sites of Glucan Synthesis

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Six sites of 2-deoxyglucose (2DG)–induced lysis on three yeasts (Schizosaccharomyces pombe, Pichia farinosa, and Saccharomyces cerevisiae) coincided with the regions of growth of their glucan layers. Identification of the glucan layer as the site of lysis suggests a mechanism of attack by 2DG or by its derivatives. It is proposed that the glucan layer grows by addition of glucose into internal breaks of polysaccharide molecules. 2DG inhibited resynthesis (insertion of glucose) of the broken glycosidic linkage.

Recent autoradiographic studies (4, 6, 7) have shown the growth regions of the glucan layer in the cell walls of three different yeasts. Other investigators have suggested that 2-deoxyglucose (2-deoxy-D-arabino-hexose, 2DG) prevents yeast growth by sequestering uridine nucleotides and thus it inhibits polysaccharide synthesis (1, 3). 2DG not only represses cell growth but also induces lysis (8). This paper illustrates that the sites of this lysis correspond to the regions of glucan synthesis of three yeasts. Therefore, I propose a mechanism of lysis.

MATERIALS AND METHODS

The yeasts studied were a fission yeast, Schizosaccharomyces pombe, and two budding yeasts, Pichia farinosa and Saccharomyces cerevisiae. Cultures were maintained and experiments were performed in a commercial, defined medium, supplemented with KH₂PO₄ and asparagine (5); other culturing conditions were described earlier (4, 6, 7). Addition of 2DG and photographic methods were also given (5).

RESULTS

The photographs of lysing yeast cells (Figs. 1, 2) illustrate the constant observation that 2DG–induced lysis occurred only at certain cellular regions. Thus, both ends (Fig. 1a–c) and the cell-plate region (Fig. 1d–e) of fission yeasts were sites of lysis. Budding yeasts were lysed at the tips of their buds (Fig. 2a–d), but adult forms of the bakers’ yeast (which had no buds) were also lysed (Fig. 2a–b).

S. pombe was readily lysed in suspension (7a, 8; over 50% of a population ordinarily was lysed within 1 hr) but the other two yeasts were less sensitive. However, these differences are not obvious in the photographs because all three yeasts were lysed on slides.

DISCUSSION

Observations of lysis. In every example studied, lysis occurred at regions shown by autoradiography to be the principal sites of glucan synthesis. Thus, for S. pombe, the primary growing end, the secondary growing end, and the cell-plate region were the sites of lysis as well as the principal regions of glucan synthesis of fission yeasts (4). Buds of P. farinosa and S. cerevisiae were lysed only at their tips—again the principal regions of glucan synthesis for these yeasts (6, 7). I found that lysis occurred on a few adult cells (budless) of the bakers’ yeast, and this phenomenon may reflect the glucan synthesis associated with bud initiation (7).

Characteristics of the lytic attack. Data have implied (1, 3) that 2DG inhibits growth by decreasing the cellular pool of the uridine nucleotides necessary for biosynthesis of polysaccharides; this decrease results from the accumulation of uridine diphosphate 2-deoxyglucose (UDP-2DG). However, these authors (1, 3) did not consider the now-apparent fact that more than a simple inhibition of growth occurred—i.e., the cells were killed by a lytic effect of 2DG or one of its derivatives.

To formulate a mechanism for the lytic effect, one must consider the following facts. (i) Lysis occurs only at the sites of synthesis of glucans (mentioned above). (ii) Lysis is produced only if the cell is growing (7a, 8). (iii) The rate of initia-
tion of lysis is strictly proportional to the rate of cellular extension (7a). (iv) Growth dependency is more important than the molar ratio of normal substrate to inhibitor because the lytic effect decreases through the late log phase when the amount of glucose relative to that of 2DG is decreasing (7a). (v) Cellular loss of glucan is progressive—for the entire end of the cell may be dissolved away [compare Fig. 1 with previously published photographs of advanced lysis (5, 8)]. (vi) UDP2DG accumulates in the presence of either glucose (1) or galactose (3). (vii) Any effect of the inhibition of fermentation by 2DG (2) is probably secondary.

2DG-induced lysis and the mechanism of glucan synthesis. The strong association of 2DG-induced lysis with growth, and in particular, growth of the glucan layer, suggests that 2DG is interfering in some way with the normal growth mechanism of that layer. Apparently, the dissolution of the normally insoluble glucan is mediated by enzymes, and the strong association with growth implies that the enzymes are part of the normal growth mechanism. If the glucan layer grows by mere addition of glucose to the ends of its backbone and side-chain components, and then extends by slipping along the lengthened chains, it is difficult to rationalize the effects of addition of 2DG. But if the interwoven glucan molecules are firmly bonded to one another and are incapable of slipping upon each other, then addition of glucose to the ends of the molecules would not lead to extension of the glucan layer and thus another mechanism of growth must be proposed. I suggest that the glucan layer grows by insertion of glucose from uridine diphosphate glucose (UDPG) into cuts made enzymically in pre-existing glucan molecules [similar to a mechanism proposed (9) for growth of bacterial cell walls]. According to this hypothesis, the UDP2DG which accumulates (1, 3) presumably would not interfere with the glucanases that cut glucan molecules preparatory to the insertion of glucose, but would apparently interfere with the mechanism that ordinarily restores the glucan molecules to the state of intactness. (UDP2DG cannot be presumed to activate the glucanases; otherwise lysis would occur when 2DG is added to non-growing cells.)

The mode of interference by UDP2DG with insertion of glucose from UDPG is not apparent because the accumulated UDP2DG may only

Fig. 1. Phase-contrast photomicrographs of 2DG-induced lysis of Schizosaccharomyces pombe. (a) Lysis at primary growing end. (b) Very early lysis at primary growing end. (c) Lysis at both primary and secondary growing ends of cell. (d, e) Progress of lysis at cell plate (d preceded e by 60 sec).
inhibit glucose insertion, or 2DG itself may be incorrectly inserted. (The former is preferred for its simplicity, but either event may lead to lysis.) Nevertheless, when enough of the interwoven matrix of glucan molecules has been cut at the growth site, the cytoplasm is extruded through the cut area and thereby produces the lysis. Earlier (1, 3) mechanisms proposed for 2DG effects neither took into account the progressive nature of the lysis nor the fact that lysis is the primary cytological effect.

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LITERATURE CITED


